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# THE EFFECTS OF ACUTE GAMMA IRRADIATION ON THE BRINE SHRIMP, *ARTEMIA*. I. LIFE SPANS AND MALE REPRODUCTIVE PERFORMANCE [1, 2, 3]

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The variety of metazoans which have been successfully exploited for genetic studies is quite limited, and the majority of such animals are either insects or vertebrates. The range of metazoans used in assaying the effects of radiation on total reproductive behavior has not been much greater. A broadening of the foundation upon which our knowledge of such effects is based necessitates using organisms which are less well known than the standard laboratory species.

Initial reports of Bowen (1962, 1963a, 1963b, 1964), Grosch (1962), Grosch and Erdman (1955) indicated that *Artemia* is well suited as a laboratory animal for studies of a genetic or radiobiological nature. The primary goal of the present study, the first of a series, was to estimate the overall effects of acute gamma radiation on the fitness components of amphigonic *Artemia*. Refined measurements of genetic damage have had to be postponed until after such an analysis.

The experimental design, and effects on life span and male productivity are reported in this paper. Effects on female productivity shall be the concern of a subsequent paper. This is the first and only report concerning the effects of electromagnetic radiation on male reproductive performance in *Artemia*.

## MATERIALS AND METHODS

Freshly matured brine shrimp were obtained from two stock #3 culture jars. This stock was derived from commercially obtained Californian cysts, and has been maintained in five-gallon battery jars since 1957 at the Marine Biological Laboratory, Woods Hole. The jars are kept at room temperatures and allowed to evaporate each winter. The cultures are re-established each spring by adding distilled water to the resultant brine and precipitated salts. The spring culture is thus derived from overwintering cysts and a few adults which may still be alive in the brine.

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The culture medium consisted of filtered sea water to which was added 50 g of sodium chloride (Fisher Scientific Co.) per liter (Bowen, 1962). Later, after the surviving animals were transported to Raleigh, North Carolina, "Instant Ocean" (Aquarium Systems, Inc., Wickliffe, Ohio) was substituted for the sea water.

Holding jars were set up eight days prior to irradiation, and young adults were segregated according to sex for the next two days. All adults thus obtained were then held for an additional five days. Eight treatment groups of five males each were assigned by use of a random numbers table to provide irradiated males to be mated to untreated females. This procedure was repeated for females, to provide irradiated females to be mated to untreated males. Separate randomizations then assigned an untreated mate to each experimental animal. Pint-size glass jars, each containing 10 fl. oz. of medium, were used for pair-matings.

For exposure, each treatment group was placed in a small plastic petri dish during exposure and given the appropriate dose of gamma radiation from the M. B. L. cesium-137 unit (Table I). There is no significant heat problem with this source, and room temperatures were thermostatically maintained in the middle seventies (°F)

TABLE I

*Male and female adult life spans in days, with two-tailed t test comparisons of treated* versus *control animals (maximum range of error in exposure time:* ±0.01 *min.*)

| Level | Dose (kR) | Exposure time (min.) | $\bar{X} \pm$ S.E. | t | X ± S.E. | t |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 52.9 ± 8.5 | — | 45.6 ± 6.2 | — |
| 2 | 0.1 | 0.20 | 49.0 ± 8.7 | 0.32 | 43.6 ± 5.5 | — |
| | | | | | 30.4 ± 5.9[a] | |
| 3 | 1 | 1.00 | 55.8 ± 4.2 | 0.31 | 40.9 ± 8.0 | 0.47 |
| 4 | 2 | 2.00 | 55.7 ± 5.5 | 0.28 | 28.7 ± 4.6 | 2.20 |
| 5 | 5 | 2.00 | 48.2 ± 8.0 | 0.40 | 40.4 ± 5.8 | 0.61 |
| 6 | 10 | 2.05 | 45.8 ± 5.7 | 0.70 | 26.7 ± 5.8 | 2.24 |
| 7 | 50 | 10.27 | 15.9 ± 1.2 | 4.30** | 9.9 ± 0.5 | 5.79*** |
| 8 | 100 | 20.54 | 5.9 ± 0.5 | 5.53*** | 4.6 ± 0.6 | 6.63*** |

[a] Replication 2 differs significantly from replication 1; t = 2.30.
** Significant at 0.01; ***Significant at 0.001.

All irradiation was completed in a period of three hours, and the animals were then placed in their assigned pair-mating jars. Each jar was examined daily throughout the entire life span of the treated animal. In the treated-male series, females were held for several days after their mate's death, so that any brood which was "in utero" at his death would also be scored. The dead mate of a treated animal was replaced by an adult which was comparable in age to the dead animal, but had not yet reached senility. Replacement females were unmated at the time of use. Scoring criteria consisted of debility or death of the adult animals, and the presence of either cysts or nauplii in the jar.

Female specimens of *Artemia* customarily produce a brood every three and one-half to four days. Oviparous broods (cysts) were filtered out, dried and stored on filter paper. These cysts were later counted and scored for hatchability after hydration in small stender dishes of plain Instant Ocean. Most cysts hatch within 24–48 hours after hydration, but all hatchabilities were scored daily for seven days. Viviparous broods of nauplii were counted immediately.

Broods I and IV were set up in quart jars (each containing 24 fl. oz. of brine), and raised to adulthood. These broods were then scored for morphological abnormalities, sex ratio, and the per cent survival.

Each jar was fed daily with a dilute suspension of Fleishmann's baking yeast and evaporated water was replaced by distilled water. All culture jars received constant overhead fluorescent lighting.

Manipulation of immature and adult animals was greatly facilitated by use of a commercially obtained glass meat baster, used as a giant medicine dropper.

Eleven days later, a second replicate was set up in the same manner as just described, again utilizing five animals of each sex at each of the eight dose levels.

The enormous number of cysts produced by the controls in the treated male experiments precluded a full hatchability analysis for this level, and only a portion of their encysted broods were so analyzed. Except for this parameter, full life history and reproductive data were obtained for each test animal.

## RESULTS

### *Life span*

Means and variances were first established within each replicate, and compared by two-tailed t and F tests. The mean values of two replicates were significantly different for female life span at the 100 R level, and this level was excluded from further analysis. The differences of the other means between replicates within treatment levels were not significant. Some of the differences between variances were significant. Average mean values and pooled variances (Steel and Torrie, 1960) were obtained independently for the members of each sex within each treatment level. Comparisons between treatment levels were made for each sex, using a two-tailed t test (Table I).

Exposures of 50 and 100 kR significantly reduced male and female life spans. Reductions in female life span approach significance after doses of 2 or 10 kR, and are significant at the 0.10 level. The male and female dose-effect curves are linear on a semi log plot for the doses of 10, 50 and 100 kR, although the slopes of these lines are quite different, and demonstrate the consistently greater longevity of the homogametic male.

### *Male reproductive performance*

*Number of broods.* Comparisons of the average number of fertile broods per male are shown in Table II. While 1 kR did not significantly reduce the number of broods per male, 2 kR caused an evident decrease. The differences between the results from 2, 5, and 10 kR are not significant. After 50 and 100 kR, no broods were produced. For purposes of the present study, fertility was defined as the production of nauplii from either a viviparous brood, or from cysts. The voiding of cysts does not in itself indicate male fertility, since many females are capable of depositing unfertilized cysts (which always fail to hatch). Therefore, only fertile broods were considered in this study. Complete genetic dominant lethality is not distinguishable from sperm inactivation or failure of copulation.

*Reproductive span.* The average number of days during which males were able to sire offspring was calculated per treatment level (Table II). Examination of the records of individual males indicated three distinct categories, a short reproductive span of 11.9 days, a transitional situation, and a long reproductive span of 50.4 days. The short-span animals were found at the 5 and 10 kR irradiation levels. Five of the eight fertile males given 2 kR also belonged to this group ($\bar{X} = 11.8 \pm 0.9$). The remaining three males had a long reproductive span which raised the group's average to the tabulated 28.4 days. However, two of these three males receiving 2 kR, were temporarily sterile from day 13 to day 29 or 36, after which fertility was regained. At doses below 2 kR, no sterile periods occurred and all males exhibited long reproductive spans.

TABLE II

*Average number of fertile broods and reproductive span per irradiated* Artemia *male, with t test comparisons (one-tailed) of treated* versus *control animals*

| Level | Dose (kR) | Number of fertile broods | | Reproductive span (days) | | Over-all reproductive span |
|---|---|---|---|---|---|---|
| | | $\bar{X}$ ± S.E. | t | $\bar{X}$ ± S.E. | t | X ± S.E. |
| 1 | 0 | 10.4 ± 1.9 | — | 53.7 ± 8.1 | — | 50.4 ± 3.3 |
| 2 | 0.1 | 8.2 ± 1.4 | 0.928 | 44.1 ± 6.4 | 0.932 | |
| 3 | 1 | 9.8 ± 1.1 | 0.276 | 52.0 ± 4.1 | 0.187 | |
| 4 | 2 | 4.0 ± 1.4 | 2.700* | 28.4 ± 8.7 | 2.126* | |
| 5 | 5 | 1.7 ± 0.5 | 4.416** | 11.1 ± 1.4 | 5.195** | 11.9 ± 0.7 |
| 6 | 10 | 2.2 ± 0.6 | 4.121** | 12.6 ± 1.7 | 4.951** | |

* Significant at 0.05.
** Significant at 0.01.

On day 46 of replicate 2, the three surviving males of the 5 kR exposure and four surviving males which received 10 kR were scored as aspermic by gross observation. Males at lower levels had cellular material present in their seminal vesicles. However, two of the three males which received 2 kR failed to produce additional nauplii.

Unlike the female response to radiation (data to be presented in paper II of this series) fertility in male brine shrimp appears to be unrelated to life span. A short reproductive span was not correlated with life span.

*Cyst hatchability.* Hatchability tests were conducted for all oviparous broods. However, analysis was restricted to those encysted broods which (1) were not completely infertile, (2) contained at least 25 cysts (*i.e.*, the majority), and (3) were not derived from partially viviparous broods. The percentage of hatched cysts was calculated for each brood. The arcsin $\sqrt{\%}$ transformation was then obtained for each value (Steel and Torrie, 1960), since it is not legitimate to use the untransformed values for the standard error. Untransformed treatment means and t test comparisons are given in Table III. The number of encysted broods per level which could be utilized varied considerably, and only part of the cysts produced by

TABLE III

Artemia *hatchability per fertile encysted brood, with t test comparisons of treated versus control animals. The males received an acute gamma ray dose*

| Level | Dose (kR) | % hatch |  | No. of broods scored |
|---|---|---|---|---|
|  |  | X | t |  |
| 1 | 0 | 24.3 |  | 12 |
| 2 | 0.1 | 27.4 | 0.472 | 27 |
| 3 | 1 | 15.2 | 1.583 | 49 |
| 4 | 2 | 21.4 | 0.445 | 13 |
| 5 | 5 | 13.7 | 1.366 | 3 |
| 6 | 10 | 6.5 | 3.200** | 13 |

** Significant at 0.01.

unirradiated males were scored. These control data are in close agreement with the control values obtained in the irradiated-female experiment (Squire, in preparation).

The available sample size at 5 kR is too small to warrant conclusions. Hatchabilities were significantly reduced after 10 kR. This decrease may be ascribed to: (1) failure of some eggs to be fertilized, or, (2) genetic lethals which act prior to the nauplius stage. Thus we consider cyst hatchability as a measure of "dominant" lethal events. We may then calculate the percentage of dominant lethal events as:

$$\%\ \text{DLE} = \frac{\%\ \text{control hatch} - \%\ \text{treated hatch}}{\%\ \text{control hatch}} \times 100$$

This calculation gives an approximate value of 73% for 10 kR.

*Survival to adulthood, sex ratio, and $X_1$ productivity.* Broods I and IV were scored for survival to adulthood and sex ratio. The observed values were highly

TABLE IV

*Survival to adulthood, sex ratios and morphological abnormalities of $X_1$ animals fathered by gamma irradiated male brine shrimp*

| Level | Dose (kR) | % survival to adulthood |  | No. of broods produced | Abnormalities |  | Sex ratio % males |
|---|---|---|---|---|---|---|---|
|  |  | X ± S.E. | t |  | fraction | % |  |
| 1 | 0 | 62.5 ± 4.5 |  | 14 | 1/474 | 0.2 | 54.6 |
| 2 | 0.1 | 58.3 ± 5.3 | 0.579 | 12 | 6/584 | 1.0 | 49.5 |
| 3 | 1 | 69.9 ± 2.1 | (1.370) | 14 | 3/564 | 0.5 | 52.3 |
| 4 | 2 | 65.7 ± 6.9 | (0.378) | 8 | 0/322 | 0 | 52.2 |
| 5 | 5 | 56.2 ± 2.9 | 1.094 | 7 | 6/244 | 2.5 | 53.3 |
| 6 | 10 | 28.2 ± 2.2 | 4.040** | 4 | 2/50 | 4.0 | 60.0 |
| $X^2$ comparison |  |  |  |  |  |  | 5.25NS |

** Significant 0.01.

heterogeneous and showed no obvious correlation with (1) brood size, (2) deposition as a viviparous *vs.* oviparous brood, or (3) the length of the post-irradiation period. All viviparous broods were maintained under the same conditions at the M. B. L., Woods Hole. All oviparous broods were maintained under comparable conditions at Raleigh.

Since the results did not differ greatly between broods I and IV, the data were pooled for each treatment level and analyzed on a per-brood basis using a one-tailed t test. After pooling, these values demonstrate a highly significant reduction at 10 kR, but not at lower doses. Sex ratios were not significantly altered at any dose level (Table IV).

In addition, some data were obtained for the reproduction of animals from brood I. The surviving offspring of 10 kR males died early and were usually sterile. Some of these animals were full-sib mated while others were mated to normal animals. Some were mated to normal animals after their sib died. A total of three fertile broods was produced. At 5 kR, a total of 39 full-sib matings resulted in no viviparous broods and a low frequency of oviparous broods. Adult mortality was still very high. At 2 kR, oviparous broods were more frequent, viviparous broods rare, and adult mortality high. At lower dose levels, oviparous broods were common, while viviparous broods remained relatively rare. Adult mortality was not excessive. Labor and space requirements made it impossible to conduct full life history studies for these $X_1$ animals and most cyst hatchabilities were not analyzed. Consequently, observations had best remain on a qualitative basis.

The number of morphological abnormalities in broods I and IV are summarized in Table IV. Four of the level 5 animals were females. One of the level 2 animals was too immature to sex. The other 13 animals were males. The abnormal females consisted of: (1) one with a pair of male-like claspers and an ovisac full of yolky-appearing material which did not resemble normal eggs, (2) one with a stubby tail and paired ovisacs, (3) one with an undeveloped tubular ovisac and an arched tail, and (4) one with a bulbous distal section of the ovisac. This section is normally thin and tubular, with its long axis parallel to that of the trunk. The axis of this ovisac was ventrally perpendicular to that of the trunk. Abnormalities in the males included eyes which were mosaic for normal pigmentation, shortened or absent claspers, stubby tails, missing caudal furcae, abnormal seminal vesicles, bent tails and apparently fused phyllopodia. Most of these abnormalities were bilaterally asymmetrical.

## Discussion

### *Life span*

These results suggest a threshold for obvious life span effects in the 10 kR region, but data are not available to check additional points in the 10–100 kR range against the linear semi-log plot. Ballardin and Metalli (1966) concluded that the life span values for several unspecified strains of *Artemia* may be fitted by an exponential function of the dose after acute exposure of 10, 20, 30, 40, or 50 kR rad. While the curves for diploid and tetraploid females, both bisexual and parthenogenetic, and for tetraploid males show no appreciable shoulder, that for the bisexual diploid males shows a definite shoulder; and these males are more resistant than the other animals. Their abstract does not specify the actual life span values. Grosch and Erdman (1955) also reported males to be more resistant than females, and the data indicated an obvious effect in the 10 kR region. Culture techniques were still sub-optimal in 1955, however, resulting in decreased life span values for both control and treated animals.

The assumption that adult brine shrimp lack tissues with proliferative cells is not valid. Lochhead and Lochhead (1941) found frequent mitotic figures in the hemopoietic organs, and M. Lochhead (personal communication) has observed occasional mitotic figures in the gut as well. To what extent these somatic divisions may be necessary for adult survival is not known. According to Lochhead and Lochhead (1941), hemopoietic mitoses increase in frequency after physical injury. The blood cells thus produced are phagocytic, and perhaps also perform some nutritive function in oogenesis. Conceivably these cells function in tissue replacement necessary for survival, and consequently *Artemia* is more sensitive to radiation than the adult form of the holometabolous insects.

The extent to which the nonlocalized centromere of *Artemia* chromosomes (Stefani, 1963) confers somatic cell resistance to radiation damage is not yet known. The retention of radiation-induced fragments over many cell generations has been repeatedly demonstrated in animals which have holokinetic chromosomes, although mosaic fragment loss may also occur (Barry, Guthrie and Dollinger, 1967; Hughes-Schrader and Ris, 1941; Hughes-Schrader and Schrader, 1961; Nelson-Rees, 1962; Ris, 1942; Tazima, 1964; Tazima and Onimaru, 1969). As Stefani (1963) pointed out, such a radiation study should be conducted in *Artemia*. Grosch and Erdman (1955) noted that a source of radio-resistance may be the somatic polyploidy present in some tissues.

Radiation-induced shortening of life span must be viewed as a summation of innumerable deleterious effects on the animal's physiological processes and cannot be ascribed to genetic damage alone. In this species, the female is the heterogametic sex; and it is the heterogametic sex which is usually less resistant to environmental stress and often short-lived.

### *Male reproductive performance*

*Reproductive span.* Since there are no reports concerning the effects of electromagnetic radiation on male reproductive performance in brine shrimp, we are forced to compare *Artemia* to such distant relations as the insects and mammals. Such comparisons must consider the anatomical, physiological, and genetic differences which separate such diverse organisms. In *Artemia*, spermatogenesis is continuous and its stages may occur in waves of partially synchronized activity. All stages are present in the mature male (Fautrez-Firlefyn, 1951). The sperm are aflagellate and may be amoeboid (Fautrez-Firlefyn and Fautrez, 1955). Stefani (1963) found the chromosomes to be polycentric in the species of *Artemia* which he studied. Finally, *Artemia* is aquatic rather than terrestrial.

A dose of 2 kR is enough to destroy or incapacitate the spermatocytes of most organisms. While this dose may allow some sperm cells to be formed, most of these spermatozoa carry dominant genetic defects which cause zygotic death early in embryonic development. In the present study which requires decisions based upon interrupted cyst deposit and failure in larval emergence as well as the absence of naupliar production, sterility of the male must be assessed in terms of any cessation of visible offspring from his mate. Nevertheless 2 kR as the sterilizing dose for spermatocytes is consistent with results for some insects, for example *Bombyx* (Sado, 1961; Tazima, 1961, 1964). Diptera are more sensitive, with 1.5 kR the upper limit (Riemann, 1967). Mice may be in another class entirely since less than 700 R suffices (Davies and Evans, 1966; Mandl, 1964).

Spermatogonial response to radiation varies considerably according to the organism and the type of spermatogonial cell involved. In the mouse, 300 R destroys "type B," intermediate, and most "type A" spermatogonia. One resistant component of the "type A" population survives, and eventually multiplies to regenerate the germinal epithelium (Oakberg, 1965). A similar result occurs in *Bombyx* after 2 kR (Sado, 1961). Spermatogonial LD-50's include about 1 kR for *Bombyx* and 23 R for mice.

We conclude that the short fertility period observed in specimens of *Artemia* after 5 and 10 kR represents radio resistant cells which were sperm and perhaps spermatids at the time of treatment. Since the reproductive pattern for most 2 kR males is the same as that for higher doses, the same conclusion seems justified for this level as well. The presence of cellular material in some seminal vesicles of 2 kR males 46 days after treatment, the temporary sterility of two 2 kR males, and the lack of sterility of one 2 kR male all suggest spermatogonial regeneration at this treatment level. The failure of some of these males to regain fertility may be due either to high genetic dominant lethality or to low sperm concentration. The latter could result from incomplete testis replenishment by spermatogonia (Gillette, Hopwood, Carlson and Gassner, 1964). This replenishment and subsequent spermatogenesis is believed to have obscured alteration of the reproductive pattern in the 2 kR male which lacked a sterile period. Replenishment probably had an even greater influence at 1 kR, where no sterile period was observed; also some of the primary spermatocytes may have survived destruction.

Since all surviving males at 5 kR and 10 kR levels were observed to be aspermic on the 46th day after treatment, either all "type A" spermatogonia were destroyed or testis replenishment was insignificant at these doses. The actual duration of the initial fertility period, and hence the subsequent sterility period, is a function of the rate of sperm utilization by the irradiated male. In turn this may depend upon the availability of females and their reproductive cycle. In our experiment, each male was isolated with a single female. If each male had been placed with a fresh receptive female daily, the period of fertility might have been shortened.

Furthermore, the present results were obtained with freshly matured males. Conceivably, slightly older males would contain a greater number of "type A" spermatogonia. If this is the case, more rapid testicular replenishment might result in those males.

*Cyst hatchability.* In general, spermatids are believed to be more sensitive than mature spermatozoa to the effects of irradiation (Mandl, 1964). The doses required to induce 99% dominant lethal events in the mature sperm of many insects with monokinetic chromosomes are in the range of 3 kR to 11 kR (LaChance, 1967). With holokinetic chromosomes, doses have ranged from 4 kR to 100 kR (LaChance, Schmidt and Bushland, 1967; North and Holt, 1968a, 1968b). Despite considerable differences between insects and shrimp, the estimate of 73% dominant lethality at 10 kR seems reasonable. Indeed the dose required for *Artemia* seems to be rather low for a holokinetic species, but well within the range of other reported values. Possibly treated spermatids as well as sperm contributed to this value. Such a heterogeneity would be expected to lower the observed resistance values.

The low hatchability value for the 1 kR males is probably fortuitous. Upon examination of individual male records, it was found that the average hatchability is no different for the first 15 days than it is for the remainder of the reproductive span. (The untransformed average values were 16.2% and 14.9%, respectively.) This observation rules out the possibility that the lowered value was due to irradiation effects on meiocytes and earlier stages which were excluded from analysis of the higher levels. If this had been the case, then the average values for hatchability would be expected to be lower in the second period which reflects pre-spermatid stages of treatment.

In *Artemia*, the date of brood deposition cannot be accurately correlated with the cytological stage present at the time of irradiation for several reasons: (1) The post-irradiation interval also reflects the reproductive cycle of the female, since only metaphase I eggs are sampled. (2) The normal duration of each spermatogenic stage, the effects of irradiation on the duration of each stage, and the effects of the lower doses of radiation on the survival of these stages are unknown for *Artemia*. (3) The male reproductive system consists of a pair of genital tracts without a common penis. Thus a single fertilization reflects a contribution by one of these tracts, but two successive broods may represent the same tract twice or each tract once. (4) Each testis is a long tubular organ with spermatogonia present along its periphery. A given ejaculate may therefore contain sperm which are heterogeneous in terms of the developmental stage irradiated. Such heterogeneity might be increased by delayed utilization of sperm from males not subjected to sperm exhaustion by multiple mating.

It has been tacitly assumed that the various secretory cells of the anterior testis have not been damaged. While such cells have been relatively radio-resistant in other animals, no data exist for *Artemia*. A hypothetical threshold at 2 kR for these cells would not be inconsistent with the data, if the observed effect were delayed.

*Survival to adulthood, sex ratio, and* $X_1$ *productivity.* By combining data on survival to adulthood with the information gained for the mortality and reproductive behavior of $X_1$ specimens of *Artemia*, we may draw some general conclusions. After an adult male has received 10 kR, most of his potential offspring are eliminated prior to the naupliar stage. Those which become nauplii show a pronounced mortality rate prior to maturity. Those which reach maturity continue to exhibit a high mortality rate and are almost uniformly sterile. A similar, but less pronounced pattern is indicated for the 5 kR group. Morphological abnormalities are relatively common in both of these groups. The 2 kR level is notable for its lack of demonstrable effect on hatchability, survival to adulthood, or morphological abnormalities. Effects were still observed in the adult $X_1$ of 2 kR males in terms of reproductive behavior and mortality.

The mosaic nature of morphological abnormalities has often been ascribed to alteration of one-half of a DNA helix following sperm irradiation in other organisms. We cannot restrict ourselves to this interpretation in *Artemia*. The holokinetic nature of the *Artemia* chromosome is expected to reduce fragment loss following irradiation. However, small fragments may still disappear from some cell lines, and thus produce a mosaic individual. Recently, Tazima and Onimaru (1969) reached the same conclusion in *Bombyx* where an exponential increase in mosaic frequency was observed in a dose-effect study of irradiated sperm. An earlier report of mosaic fragment loss is that of Nelson-Rees (1962). Incomplete penetrance of induced mutations might also be reflected in asymmetry.

The data for the 10 kR level suggest that the $X_1$ is much less fertile than are the irradiated males themselves, a sterility pattern typical of species with holokinetic chromosomes. Investigated cases have involved exposure of post-meiotic stages, often mature sperm, to sterilizing doses of radiation. Since the effect of single-break aberrations is minimized by the holokinetic nature of the chromosome, the sterility pattern is believed to reflect multi-break phenomena, and in particular, translocations (Bauer, 1967; Nordenskiold, 1963; North and Holt, 1968a, 1968b; Walker and Quintana, 1968). Irregular fragment behavior and inversion heterozygosity may be additional factors, and Nordenskiold (1963) found in the plant *Luzula* that if a holokinetic chromosome is fragmented into more than two pieces, subsequent recombination with an unfragmented homologue will yield a secondary fragmentation of that homologue. Meiotic segregation may then result in chromosomal imbalance and sterility.

The failure to detect a significant alteration in sex ratio may be due either to a reduced sample size at 10 kR, or to an insufficiently large dose of radiation. Grosch (1962) observed that the sex ratio of *Artemia* tended to favor males when the parents were of irradiated ancestry. This tendency was especially pronounced in populations which were on the verge of extinction. Grosch correlated this effect with female heterogamety.

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## Summary and Conclusions

### *Life span of both sexes*

Freshly matured male and female specimens of Californian *Artemia* (stock #3) were irradiated with acute doses of 0, 0.1, 1, 2, 5, 10, 50, or 100 kR gamma radiation and pair-mated to untreated animals. Significant reductions in life span resulted after 50 or 100 kR to either sex. Reductions in female life span approached significance after 10 kR. This moderate radiosensitivity can be explained if cell proliferation in some adult tissues improves the probability of individual survival.

### *Male reproductive performance*

Treated males were completely sterile after doses of 50–100 kR. Males which received doses of 5 or 10 kR had an initial fertility period lasting 12 days, followed by permanent sterility. After a dose of 2 kR, 7 out of a sample of 8 males demonstrated the same initial period of fertility, followed by a period of sterility. Two of the 7 subsequently recovered their fertility. No sterile period occurred after 0.1 or 1 kR. These results are interpreted as indicating spermatogonial stem-cell replenishment of the testicular epithelium in some males after 2 kR and in all males irradiated with 1 kR. It is further hypothesized that all spermatocytes were in some way incapacitated or destroyed following doses above 2 kR.

Hatchability data failed to detect induced dominant lethality in sperm (and perhaps spermatids) after 2 kR. Induced damage resulted in 73% dominant lethal events at 10 kR. No effect on other stages was observed at less than 2 kR.

In the $X_1$ generation, a dose of 10 kR reduced survival to adulthood, fertility, and adult life span. Similar, but less pronounced effects were obtained after 2 and 5 kR. These data indicate induced genetic damage. No significant alterations were observed in sex ratios.

$X_1$ sterility appeared to be greater than that observed for irradiated sperm. This point is being reinvestigated, and would be compatible with the reported holokinetic nature of the *Artemia* chromosome.

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